In a non-diabetic Japanese population, the combination of macroalbuminuria and increased urine beta 2-microglobulin predicts a decline of renal function: the Takahata study

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Abstract

Background. Glomerular and tubular damage are important factors in the development of renal insufficiency. However, the interaction of these factors is largely unknown in the non-diabetic Japanese population. To clarify the relationship between renal insufficiency and both glomerular and tubular damage, we conducted a community-based study using albuminuria and urine beta 2-microglobulin as markers of glomerular and tubular damages, respectively.

Methods. Subjects of this study were 2816 non-diabetic individuals >40 years old in Takahata, Japan. The urine albumin–creatinine ratio (UACR) and urine beta 2-microglobulin–creatinine ratio (UBCR) were assessed from single spot urine. The glomerular filtration rate (eGFR) was estimated using the abbreviated MDRD equation with a Japanese coefficient.

Results. The prevalence of albuminuria (UACR >20 mg/g in men and >30 mg/g in women), increased UBCR (>300 µg/g) and renal insufficiency (eGFR <60 mL/min/1.73 m²) were 21.0%, 12.5% and 21.7%, respectively, and there was only a small overlap between the three. The mean eGFR was significantly lower in subjects with macroalbuminuria (UACR >200 mg/g in men and >300 mg/g in women) and increased UBCR. No urinary abnormalities were observed in 71.7% of the 611 subjects with renal insufficiency, and were more common in young, women and the non-hypertensive population. The 1-year decline of eGFR was greatest in subjects with an overlap of macroalbuminuria and increased UBCR.

Conclusions. This study indicated that only a small part of renal insufficiency accompanied increased urine albumin or beta 2-microglobulin in the non-diabetic Japanese population. The combination of macroalbuminuria and increased urine beta 2-microglobulin might predict faster renal deterioration.

Keywords: albuminuria; beta 2-microglobulin; general population; renal insufficiency

Introduction

Chronic kidney disease (CKD) is getting an attention as a worldwide problem and there is now an urgent need for the prevention of this disease. To this end, new practice guidelines for CKD have been advocated [1]. According to the criteria, albuminuria and renal insufficiency [glomerular filtration rate (GFR) <60 mL/min/1.73 m²] are categorized as an early stage (stage 1–2) and advanced stage (stage 3–5) of CKD, respectively.

Albuminuria is a marker of glomerular damage and is followed by renal impairment, both in the diabetic and non-diabetic population [2,3]. However, in clinical practice, not all patients with renal insufficiency demonstrate albuminuria. In diabetic nephropathy, it has been reported that at least 20% of renal failure occurs via a non-albuminuric pathway [4]. Furthermore, Garg et al. showed that albuminuria and renal insufficiency identify different segments in the general population [5]. These reports suggest that renal insufficiency does not always originate from the albuminuric pathway.

Renal tubulointerstitial damage can be an alternative pathway to renal insufficiency [6]. However, the significance of this pathway in the progression of CKD is unclear in the general population. Therefore, to clarify the relationship between renal insufficiency and glomerular and tubular damage, we conducted a community-based study using albuminuria and increased urine beta 2-microglobulin as markers of glomerular and tubular damage, respectively, in the general Japanese population.
Methods

Study population
This study is part of the ongoing Molecular Epidemiological study utilizing the Regional Characteristics of 21st Century Centers of Excellence (COE) Program in Japan. Details of the study methodology have been described elsewhere [7].

This study utilized a community-based annual health checkup that invited all inhabitants over the age of 40 years in Takahata town (total population 26 026), located in the northern part of Japan. This region has a resident population of 15 222 adults over the age of 40 years (7109 males and 8113 females). From June 2004 through to November 2005, 1394 men and 1771 women (total 3165) took part in the program and agreed to join the study. The participation rate was 20.8%. The participants were thought to be generally healthy. This study was approved by the Institutional Ethical Committee and all participants gave written informed consent.

Fifty subjects were excluded from the analysis due to incomplete data. To avoid the effects of known renal disease, we excluded 59 subjects with a past or current history of renal disease and 240 diabetic subjects. A total of 2816 subjects entered into the final statistical analysis. There were 1222 (44.3%) men and 1594 (55.7%) women. The mean age was 63 years.

We examined the change of estimated GFR (eGFR) by the levels of albuminuria and urine beta-2 microglobulin over a 1-year period. In a longitudinal observation, 1361 subjects who attended the study, both in 2004 and 2005, were analysed. There were 594 (43.6%) men and 767 (56.4%) women. The mean age was 64 years. The baseline characteristics of the subjects in the longitudinal observation were similar to those of the total subjects.

Measurements
Subjects used a self-reported questionnaire to document medical history, current medication and clinical symptoms. Systolic and diastolic blood pressures were determined by using a mercury manometer in a sitting position after at least 5-min rest. Hypertension was defined as a systolic blood pressure ≥140 mmHg and/or a diastolic blood pressure ≥90 mmHg and/or the use of anti-hypertensive medication. Obesity was specified as a body mass index ≥25.0 kg/m², both in men and women [8]. Diabetes was ascertained either by self-reported physical diagnosis or by a measure of plasma glucose levels ≥126 mg/dL or a HbA1c value ≥6.5%. The Urine albumin–creatinine ratio (UACR) was calculated from a single spot urine specimen collected in the early morning. Urine albumin concentrations were determined by immunoturbidimetry. Albuminuria was defined as a UACR ≥20 mg/g in men and ≥30 mg/g in women. Microalbuminuria was defined as a UACR 20–200 mg/g in men and 30–300 mg/g in women. Macroalbuminuria was defined as a UACR >200 mg/g in men and >300 mg/g in women [1]. Normalalbuminuria was defined as a UACR <20 mg/g in men and <30 mg/g in women and was divided into two subgroups defined as low normal (UACR <10 mg/g in men and <15 mg/g in women, respectively) and high normal (UACR 10–19 mg/g in men and 15–29 mg/g in women, respectively). Urine beta-2 microglobulin was assessed using the latex agglutination method (BML, Inc. Tokyo, Japan). Urine beta-2 microglobulin levels corrected with urine creatinine [urine beta-2 microglobulin–creatinine ratio (UBCRR)] were used for all analyses. Serum creatinine (SCr) was measured using an enzymatic method. The GFR was estimated using the abbreviated Modification of Diet in Renal Disease (MDRD) study equation with the Japanese coefficient, 0.881. To estimate GFR, we used calibrated SCr values obtained with the following formula: SCr (Yaffe method) = 0.2 + SCr (enzymatic method) [9]. Twenty-four-hour urinary excretion of sodium was estimated by Kawasaki’s equation using a spot urine specimen [10].

Statistical analyses
The student’s t-test was used to evaluate differences in means, and the chi-square tests were used to evaluate differences in proportions. The non-parametric Mann-Whitney U-test and Kruskal-Wallis test were utilized for the parameters of the data that were not normally distributed. Data are expressed as the mean ± SD. All statistical analyses were performed using the software of Stat View version 5 (SAS Institute Inc, Cary, NC, USA). A significant difference was defined as P < 0.05.

Results

Baseline characteristics of participants
Baseline characteristics of the 2816 participants who entered into the final analysis are shown in Table 1. There were 1524 subjects (54.1%) with hypertension, 821 subjects (29.2%) with obesity, 954 subjects (33.9%) with hypercholesterolaemia and 590 subjects (21.0%) with albuminuria. Systolic and diastolic blood pressure, SCr, estimated 24-h urinary sodium excretion, fasting blood sugar, uric acid, eGFR and urine creatinine, percentage of current smoking and drinking were significantly higher in men compared with women. The percentage of hypercholesterolaemia was higher in women compared with men.

Distribution of eGFR
The eGFR showed normal distribution curves in this population, both in the men and women (Figure 1). The mode values of eGFR were 70–79 mL/min/1.73 m² in men and 60–69 mL/min/1.73 m² in women. In total, the percentages of subjects with eGFR >90 and <60 mL/min/1.73 m² were 3.1 and 21.7%, respectively. Renal insufficiency (eGFR <60 mL/min/1.73 m², n = 611) was mostly observed in subjects >60 years of age [47 (7.7%) in the 40–49 years age group, 91 (14.9%) in the 50–59 years age group, 217 (35.5%) in the 60–69 years age group and 256 (41.9%) in the 70 years and over age group].
We first examined the relationship between renal parameters and age (Table 2), and showed that the prevalence of renal insufficiency, albuminuria, macroalbuminuria and increased UBCR (>300 µg/g) increased with age.

We then examined the relationship between glomerular or tubular damage and renal insufficiency. To exclude the effects of major confounders such as age, gender, body mass index and systolic blood pressure, we adjusted eGFR in a general linear model. Figure 2 shows the individual data of eGFR as a function of increased UACR (A) and UBCR (B). Both graphs reveal a slight, but significant, decline of the eGFR with an increase in the UACR and UBCR.

We thirdly compared the average levels of eGFR by categorizing the UACR and UBCR into several groups. Figure 3 shows a tendency for the average levels of eGFR to decrease with increased albuminuria (A). Similarly, the average levels of eGFR decreased with increased UBCR (B). These results indicate that an increased UACR and UBCR are associated with reduced renal function.

To examine the relationship of albuminuria and increased UBCR with renal insufficiency, we performed multivariate logistic regression analysis including age, gender, body mass index and systolic blood pressure (Table 3). Only the combination of macroalbuminuria and increased UBCR (>300 µg/g), but neither of these alone, was an independent factor for renal insufficiency (<60 mL/min/1.73 m²). The eGFR was lower in those subjects with an overlap of macroalbuminuria and increased UBCR than those with either of these alone (average eGFR; 63.2 ± 17.2 versus 70.3 ± 12.2 and 67.2 ± 11.5 mL/min/1.73 m², respectively).

The prevalence of albuminuria (UACR ≥20 mg/g in men and ≥30 mg/g in women), macroalbuminuria (UACR >200 mg/g in men and >300 mg/g in women), increased UBCR (>300 µg/g) and renal insufficiency (eGFR <60 mL/min/1.73 m²) was 21.0%, 1.9%, 12.5% and 21.7%, respectively. However, only a small number of the total subjects had two or more simultaneous symptoms. Among the 611 subjects with renal insufficiency, albuminuria and macroalbuminuria were observed in only 128 (20.9%) and 18 subjects (2.9%), respectively. An increased UBCR (>300 µg/g) was observed in only 91 subjects (14.9%) with renal insufficiency. The majority of subjects (71.7%) demonstrated none of these urinary abnormalities. We further examined the characteristics associated with renal insufficiency accompanying no urinary abnormalities. This occult renal insufficiency appeared more common in young, women and subjects without the traditional risk factors of hypertension, obesity and hyperuricaemia.

One-year change of eGFR associated with UACR and UBCR

To clarify the effects of urinary abnormalities on renal deterioration, we examined the 1-year change of eGFR in association with the UACR and UBCR (Figure 4). We showed that the 1-year decline of eGFR was significantly associated with urinary abnormalities and was greater in subjects with an overlap of macroalbuminuria and increased UBCR, compared with either of these alone (mean values; −9.01 versus

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**Table 1. Comparisons of baseline characteristics between men and women**

<table>
<thead>
<tr>
<th></th>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>1222</td>
<td>1594</td>
</tr>
<tr>
<td>Age (years)</td>
<td>63.0 ± 10.4</td>
<td>62.6 ± 10.3</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>135.8 ± 16.0*</td>
<td>132.5 ± 16.1</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>81.8 ± 10.1*</td>
<td>77.3 ± 9.8</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>23.4 ± 2.9</td>
<td>23.4 ± 3.4</td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>57.4*</td>
<td>51.6</td>
</tr>
<tr>
<td>Obesity (%)</td>
<td>29.5</td>
<td>28.9</td>
</tr>
<tr>
<td>Smoker (%)</td>
<td>62.8*</td>
<td>8.8</td>
</tr>
<tr>
<td>Drinker (%)</td>
<td>72.8*</td>
<td>15.0</td>
</tr>
<tr>
<td>Hypercholesterolaemia (%)</td>
<td>23.7*</td>
<td>41.7</td>
</tr>
<tr>
<td>Serum creatinine (mg/dl)</td>
<td>0.77 ± 0.14*</td>
<td>0.59 ± 0.11</td>
</tr>
<tr>
<td>Serum total cholesterol (mg/dl)</td>
<td>193.4 ± 30.0*</td>
<td>207.2 ± 30.9</td>
</tr>
</tbody>
</table>

*Estimated 24-h urinary sodium excretion (mEq/day) 
†Estimated 24-h urinary sodium excretion from the spot urine.

**Fig. 1. The distribution of estimated GFR in men and women.**

**Relationship between albuminuria, urine beta 2-microglobulin and renal insufficiency**

We first examined the relationship between renal parameters and age (Table 2), and showed that the prevalence of renal insufficiency, albuminuria, macroalbuminuria and increased UBCR (>300 µg/g) increased with age.
Table 2. The comparison of renal damages among different age groups

<table>
<thead>
<tr>
<th>Age</th>
<th>N</th>
<th>eGFR (ml/min/1.73 m²)</th>
<th>eGFR &lt;60 (%)</th>
<th>Albuminuria (%)</th>
<th>Macroalbuminuria (%)</th>
<th>UBCR (µg/g)</th>
<th>UBCR &gt;300 µg/g (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>40–49</td>
<td>334</td>
<td>71.7 ± 9.9</td>
<td>14.1</td>
<td>11.1</td>
<td>0.3</td>
<td>86 (8.5–2466)</td>
<td>4.5</td>
</tr>
<tr>
<td>50–59</td>
<td>707</td>
<td>71.5 ± 10.4</td>
<td>12.9</td>
<td>14.1</td>
<td>0.7</td>
<td>104 (5.8–15 048)</td>
<td>7.8</td>
</tr>
<tr>
<td>60–69</td>
<td>938</td>
<td>68.2 ± 10.5</td>
<td>23.1</td>
<td>21.5</td>
<td>2.3</td>
<td>113 (8.3–4944)</td>
<td>12.3</td>
</tr>
<tr>
<td>70≤</td>
<td>837</td>
<td>64.6 ± 11.4</td>
<td>30.6</td>
<td>30.0</td>
<td>3.0</td>
<td>140 (8.4–19 097)</td>
<td>19.8</td>
</tr>
</tbody>
</table>

eGFR, estimated glomerular filtration rate; UBCR, urine beta 2-microglobulin–creatinine ratio.
Albuminuria, UACR >20 mg/g in men and >30 mg/g in women; macroalbuminuria, UACR >200 mg/g in men and >300 mg/g in women.
UBCR is expressed as median with range (min–max).

Fig. 2. The relationship between UACR (A), UBCR (B) and estimated GFR. UACR, urine albumin–creatinine ratio; UBCR, urine beta 2-microglobulin–creatinine ratio; GFR, glomerular filtration rate. Estimated GFR was adjusted for age, gender, body mass index and systolic blood pressure in a general linear model.

Fig. 3. The estimated GFR associated with albuminuria (A) and UBCR (B). Low normal albuminuria, UACR <10 mg/g in men and <15 mg/g in women; high normal albuminuria, UACR 10–19 mg/g in men and 15–29 mg/g in women; microalbuminuria, UACR 20–200 mg/g in men and 30–300 mg/g in women; macroalbuminuria, UACR >200 mg/g in men and >300 mg/g in women; UACR, urine albumin–creatinine ratio; UBCR, urine beta 2-microglobulin–creatinine ratio; GFR, glomerular filtration rate. Estimated GFR was adjusted for age, gender, body mass index and systolic blood pressure in a general linear model. Error bars represent the 95% confidence intervals.
Combination of glomerular and tubular damage

Table 3. Odds ratio for renal insufficiency (eGFR < 60 ml/min/1.73 m²)

<table>
<thead>
<tr>
<th></th>
<th>Unadjusted</th>
<th>P-value</th>
<th>Adjusted</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR (95% CI)</td>
<td></td>
<td>OR (95% CI)</td>
<td></td>
</tr>
<tr>
<td>Albuminuria</td>
<td>1.00 (0.80–1.24)</td>
<td>NS</td>
<td>0.82 (0.65–1.04)</td>
<td>NS</td>
</tr>
<tr>
<td>Macroalbuminuria</td>
<td>1.88 (1.06–3.35)</td>
<td>0.0313</td>
<td>1.59 (0.88–2.88)</td>
<td>NS</td>
</tr>
<tr>
<td>UBCR &gt;300 µg/g</td>
<td>1.31 (1.01–1.69)</td>
<td>0.0404</td>
<td>1.12 (0.86–1.46)</td>
<td>NS</td>
</tr>
<tr>
<td>Macroalbuminuria and UBCR &gt;300 µg/g</td>
<td>2.80 (1.35–5.79)</td>
<td>0.0056</td>
<td>2.24 (1.06–4.73)</td>
<td>0.0353</td>
</tr>
</tbody>
</table>

eGFR, estimated glomerular filtration rate; OR, odds ratio; UBCR, urine beta 2-microglobulin–creatinine ratio; NS, not significant.

*Adjusted for age, gender, body mass index and systolic blood pressure.

Discussion

This study revealed that only a small part of renal insufficiency accompanied albuminuria or increased beta 2-microglobulin in a non-diabetic Japanese population. The combination of macroalbuminuria and tubular damage might be a useful marker for a rapid decline of renal function.

We showed that eGFR decreased in association with an increase in the UACR. Pinto-Sietsma et al. reported a parabolic pattern between these two factors in a European non-diabetic population [3]. In our population, a similar parabolic pattern, with higher values in high normal albuminuria and microalbuminuria, was observed in an unadjusted model. However, it changed to a linear pattern after adjustment for age, gender, body mass index and blood pressure. This suggests that an increase in GFR observed in subjects with high normal albuminuria and microalbuminuria may be induced by other confounders in this population. The relationship between the UBCR and renal function was similar. Those subjects with an increased UBCR, showed a decreased eGFR. Generally, increased levels of urine beta 2-microglobulin indicate renal tubular damage [6]. Accordingly, this study revealed that renal tubular damage was related to reduced renal function. However, the increased UBCR was detected in only a small percentage (14.5%) of those with renal insufficiency. The majority of renal insufficiency could not be explained by renal tubular damage alone.
A previous report from a European population shows that macroalbuminuria, but not microalbuminuria, is a predictor of renal deterioration [11]. In Japan, Iseki et al. showed that proteinuria increased the risk of end-stage renal disease [12]. We further revealed that the interaction of tubular damage, a different segment of renal injury, could induce a much greater decline of eGFR than macroalbuminuria alone. Although albuminuria and increased UBCR were often observed in this population, neither of these alone was significantly associated with renal insufficiency. Only the interaction between them might be associated with renal insufficiency and a faster decline in renal function. It is reasonable that concomitant damage in different segments of the nephron may induce rapid renal deterioration. Concerning the interaction between proteinuria and tubular damages, Bazzi and Branten previously reported that tubular damage together with massive proteinuria could be a marker for the future development of renal failure in several types of glomerulonephritis [13,14]. Our study reveals the same phenomenon, even in a general population with less proteinuria. This finding may significantly assist the clinician in narrowing down a large number of high-risk subjects for renal deterioration, to a much smaller number.

The prevalence of renal insufficiency was high (22%) in this non-diabetic population of >40 years of age. Of note, a large percentage of those with renal insufficiency had no albuminuria. This data are consistent with the NHANES III data in a US population indicating that this finding is common in the general population, irrespective of ethnicity [5]. We also showed that the interaction between glomerular and tubular damage may be an independent risk factor for reduced renal function and may explain a proportion of occult renal insufficiency. However, the cause of the majority of renal insufficiencies in general Japanese population still remains unknown.

Several mechanisms, including ischaemic vascular disease, interstitial fibrosis, senescence of the kidney and specific medication are speculated to be involved in non-albuminuric renal impairment [4,5]. An autopsy-based population survey suggested that aging-related subclinical, renal vascular changes are associated with renal dysfunction [15]. However, in this study, renal insufficiency without urinary abnormalities was mainly observed in the young and relatively healthy subpopulation. Thus, it is unlikely that ischaemic vascular disease is the primary cause of occult renal insufficiency in this population. Brenner et al. suggested that the number of nephrons at birth might subsequently affect renal function [16]. A recent genomewide linkage analysis showed that renal function is heritable in community-based samples [17]. Further, Pinto-Sietsma et al. reported that genetic polymorphisms of endothelin-1 are associated with renal insufficiency, but not urinary albumin excretion [18]. Thus, congenital and genetic predisposition may have an impact on renal function without affecting urinary albumin excretion.

Several limitations of this study should be pointed out. Firstly, the follow-up period (12 months) is too short to reach a final conclusion on the effect of urinary abnormalities on a long-term renal prognosis. We thus aim to follow-up this cohort for several years. Secondly, there are several reports suggesting the possible influence of heavy metal poisoning, such as cadmium, on renal tubular damage [19,20]. However, we have no information on these environmental factors or specific medications, including non-steroidal, anti-inflammatory drugs, which may affect the results. We could not identify the aetiology of the renal tubular damage. Thirdly, although we used urine beta 2-microglobulin as a marker of renal tubular damage, it is unstable at low pH [6] and we have no data as to how many urine samples were at a low pH. Thus, there is a possibility that the actual number with increased UBCR may have been higher. Urine alpha 1-microglobulin is stable over a greater pH range and may be a more reliable marker for tubular damage [6]. However, the cost of using alpha 1-microglobulin is far greater than beta 2-microglobulin; thus it may not be suitable for mass screening. Fourthly, the participation rate of this study was low (20.8%), because the younger generation was covered by other health check-ups conducted at the workplace. Furthermore, to avoid the effect of known renal diseases on the results, we also excluded 59 subjects with known renal diseases. Therefore, the application of our results could be limited to the generally healthy, elder population.

In conclusion, this study revealed that a large number of subjects with renal insufficiency demonstrated no signs of glomerular and tubular damage in a non-diabetic Japanese population. The combination of macroalbuminuria and beta 2-microglobulin may predict a faster renal deterioration.

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Conflict of interest statement. None declared.


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